The 3rd APEC Workshop on Sustainable Agricultural Development
16th to 22nd November 2003, Chinese Taipei

A Strategy to Maximize Maize Production while Reducing Nitrate Contamination to the Environment

Chia-Yin Tsai

Institute of Botany
National Taiwan University, *Chinese Taipei*

A Strategy to Maximize Maize Production while Reducing Nitrate Contamination to the Environment

Chia-Yin Tsai

Institute of Botany
National Taiwan University

Introduction

Plant nutrition, along with moisture, temperature, light intensity, and pest management interacts with the genetic composition of hybrids to determine maize grain production. However, nutrient stress especially from a deficiency of nitrogen (N) is the most frequent limitation for vegetative growth and grain yield. N-sufficiency during the growing season is important not only because N plays an important role in structural and metabolic functions, but also because it affects grain fill kinetics (Tsai et al., 1990). In order to meet N requirements for maximum kernel development, the plant must either take up most of its required N during the stage of vegetative growth, or maintain its ability to absorb inorganic N (maintained in an available form in the soil) throughout the entire growing season. However, maize hybrids vary substantially in their abilities to take up and utilize N nutrition during the growing season (Tsai et al., 1991). In addition, they also differ in yield potential.

Some of the areas covered in this presentation include: 1) effects of N sink on dry matter accumulation in maize kernels, 2) predictions of grain yield potential for maize hybrids based on the N sink capacity, 3) differences among maize hybrids in nitrogen uptake and utilization, and 4) genetic variations in the ability of maize inbreds to utilize ammonium (NH_4^+) nutrition. A better understanding of these areas should be helpful to come up with an efficient N management program and breeding strategy to increase grain yield while reducing nitrate ($N0_3^-$) contamination.

Effects of N sink on dry matter accumulation in maize kernels

Amino acids and sugars are two major nutrients required for the development of maize kernels. Movements of these two nutrients into kernels are interrelated (Lyznik and Tsai, 1989), and the movement of sugars was stimulated by amino acids (Tsai et al., 1990). Therefore, an ability to continuously deposit amino acids as proteins is an important mechanism to facilitate the movement of sugars into kernels. These nitrogenous compounds are stored temporarily in leaves and stalk during the period of vegetative growth (Tsai et. al., 1991). As the kernel develops, proteins in vegetative tissues are hydrolyzed and amino acids are transported into the kernel for its development. Tsai et al. (1978; 1980; 1983) proposed that zein and glutelin function as N sinks for the

deposition of these nitrogen compounds. They thereby prevent the accumulation of deleterious levels of free amides and ammonium. The accumulation of free amino acids in the kernel due to a small N sink capacity may generate a more negative osmotic potential that favors water movement but reduces solute, such as sugars, movement into the kernel. Thus, the yield response of grain to N fertilizer is enhanced by the presence of a large N sink in the kernel (Tsai et al., 1978; 1980; 1983).

Although all proteins in the kernel may function potentially as N sinks to the extent that their synthesis reduces the accumulation of free amino acids, zein and glutelin are considered to be the major N sinks. This is so because combined they contain more than 90% of the total N in the endosperm and 60% of the total N in maize plant at maturity (Tsai et al., 1980). Unlike glutelin, however, zein is the major storage protein, and its synthesis can be manipulated readily by N fertilization as well as by genetic means. Thus, increased N in the kernel due to N fertilization is confined primarily to zein, and nonzein protein i.e., albumin, globulin, and glutelin, increases only sightly (Tsai et al., 1978; 1980). This suggests that zein plays the primary functional role as a N sink in the kernel. The positive correlation between zein content, kernel weight, and grain yield reported by Tsai et al. (1978) indicates the potential of this system.

Using the much reduced zein content of the *o2* mutant compared to its isogenic normal counterpart as a model system, Tsai et al. (1980; 1983) showed the importance of zein in affecting the movement of photosynthetic assimilates into the kernel. Since *o2* is recessive and *o2* kernels can be readily distinguished from normal kernels on the same ear, utilization of a heterozygous plant for both *o2* and normal alleles should remove interplant variability and environmental interactions which could otherwise confound this type of study. $^{14}CO_2$ was supplied to heterozygous plants (B14*o2*xB37) at 26, 39, and 45 days post pollination to monitor the movement of radioactive sugars into kernels (Tsai et al., 1980). Under high N fertilizer conditions, these three stages represent early phase, leveling off, and termination of zein synthesis, respectively, for the homozygous *o2* in hybrid backgrounds. Normal kernels, on the other hand, had a higher rate of zein accumulation than *o2* during endosperm development and terminated zein synthesis at about 55 days post pollination. Under a high N condition (201 kg N/ha), the difference in radioactivity incorporated into *o2* and normal kernels was obvious as early as 26 days post pollination. At this early stage of development, *o2* kernels contained about 93% of the radioactivity found in normal kernels. The differences were more apparent as the synthesis of zein terminated in *o2*. The *o2* kernels contained 86% of radioactivity found in normal kernels at 39 days post pollination and only 54% at 45 days (Table 1). These results are consistent with the observation that the early termination of zein synthesis in the *o2* results in a decreased rate of dry matter accumulation in the kernel thereafter (Tsai et al., 1978). Under low N conditions, like *o2*, zein accumulation in normal endosperms was substantially reduced, and the amount of radioactive photosynthates incorporated into *o2* kernels was only slightly lower than incorporated into the normal kernels on the same ear, i.e., *o2* kernels

had about 95% of the radioactivity found in normal kernels at 39 days post pollination and 88% at 45 days.

The reduction of sugar movement into the kernel, as affected by the termination of zein accumulation or by a reduced N sink capacity, correlated well with a low osmotic potential observed in the mutant. At 39 days post pollination, *o2* kernels from a segregating ear had an osmotic potential of -8.2 bars as compared to only -4.4 bars for the segregating normal kernels (Table 1), a situation similar to that of the sucrose accumulation in the endosperm of *sh2* and other starch-deficient mutants where the accumulation of high sucrose content also generated a low osmotic potential to prevent the movement of free amino acids into the kernel (Lee and Tsai, 1985). Thus, the movement of carbon (C) and nitrogen into the kernel is interrelated and is under the influence of osmotic regulation. This more negative osmotic potential of the *o2* kernel than its normal counterpart also provides an explanation that all high-lysine mutants retain higher moisture content at maturity (Lambert et al., 1969). The dynamic response of zein synthesis to N fertilizer (which is essential to increasing productivity), coupled with the observation that the early termination of zein synthesis in the *o2* mutant under high N conditions results in a reduction of sugar movement into the kernel, suggest that zein is an effective N sink to regulate dry matter accumulation in the kernel, a major determinant for grain yield.

Predictions of grain yield potential for maize hybrids based on the N sink capacity

With the cost-price squeeze felt throughout the agricultural industry, the ability to predict maximum grain yield potential and N fertilizer requirements for maize hybrids should provide a critical management tool to increase production efficiency and thereby enhance energy utilization and total productivity. As indicated from the studies shown above, the kernel sink is a primary yield determinant for maize. Other studies also provide a similar conclusion that include: 1) the movement of sugars into the kernel is substantially reduced when the deposition of nitrogenous compounds in the kernel terminates (Tsai et al., 1980), 2) restoration of normal zein accumulation in a sink-defective type kernels allows the kernel to regain a normal dry matter accumulation (Tsai et al., 1983), 3) modification of the kernel sink in an early maturing hybrid with a short duration of grain filling (by receiving pollen from a full season hybrid) not only prolongs the grain filling period until much later in kernel development but also results in a much greater kernel weight (Tsai and Tsai 1990), 4) grain yield of a given hybrid is highly correlated with its ability to deposit nitrogenous compounds in the kernel N sink as induced by N fertilizer (Tsai et al., 1980; 1983; 1992), and 5) the movement of carbon and nitrogen into maize kernels is interrelated and subject to osmotic regulation (Tsai, 1983). Based on this information, I propose that the maximum yield potential of any

given hybrid can be predicted based on measurements of the C/N ratio and the N sink capacity in the kernel using two tangible parameters, i.e. 1) kernel weight, and 2) percent protein obtained at any level of N fertilizer .

Maize hybrids were grown at the Purdue Agronomy Farm on Chalmers silty clay loam soil. Replicated field plots (4 times) were spring-treated with 0, 67, 134, 201, 268, and 447 kg N/ha of anhydrous ammonia prior to planting. Residual N in the experimental plots was cropped out a year before the study. Under this condition, the zero N plot averaged about 15 kg N/ha of residual N plus mineralized soil N each year. Each main plot consisting of rows 76 cm apart and 23 long, was separated from the others by two buffer rows. Hybrids were randomly assigned to main plots and plant density was about 54,000 plants/ha. At maturity, ears were harvested by hand, shelled, moisture percentage of the grain determined, and yield calculated. Samples were drawn with a grain probe from the shelled corn and air-dried prior to analysis for 100-kernel weight and protein. Total N was determined by a micro-kjeldahl method and protein percentage was calculated by multiplying Kjeldahl N by a factor of 6.25. This study was conducted from 1979 to 1991.

As shown in Table 2, the grain yield of a given hybrid induced by N fertilization is highly correlated with percent protein ($r > 0.93$); even though protein content among hybrids may be negatively correlated. For pioneer 3732, percent protein and grain yield increased from zero added N to its maximum with 67kg N/ha. Protein and grain yield of B73xMo17, on the other hand, increased as the level of N fertilizer increased up to 201kg N/ha. The correlation of grain yields with percent protein agrees with previously reported zein data (Tsai et al., 1992). This supports a previous observation that percent protein in the grain may serve as a parameter for estimating amounts of fertilizer required for maximum yield (Russell and Pierre, 1980).

Although zein is considered the primary N sink, all proteins in the kernel are considered together as the kernel N sink. Since the synthesis of any protein will minimize free amino acid accumulation to prevent the lowering of osmotic potential and thereby facilitate sugar movement into the kernel (Tsai et al., 1980; 1983), the determination of total proteins, rather than zein is more practical and used as an estimation of the N sink parameter for this study.

Although movement of C and N into kernels could be influenced by source supplies (Tsai et al., 1990), the C/N ratio in the kernel of a hybrid grown under an usual condition is genetically determined (Zink, 1980). The C/N ratio can be determined by dividing kernel dry weight (mg/kernel) by percent protein. This produces a constant value, except under severe N stress conditions, indicative of the increased dry weight with each increase in percentage of protein. The higher C/N ratio obtained with zero added N was caused by severe N stress. Because the limited increases in dry weight induced by additional N are primarily in the form of starch, the C/N ratio should be calculated according to starch content rather than kernel dry weight. Since the starch content for all the hybrids tested varied only from 65% to 75% of the total kernel dry weight, an average of 70% was considered an acceptable value in these studied. The C/N ratio thus

calculated appears to be hybrid-specific (Table 3) and remain nearly constant during the years tested. It thus permits the prediction of kernel weight for any given percentage of protein produced. The C/N ratio of the B73xMo17 hybrid is 26 when grown with 137 kg N/ha or more of N fertilizer (Table 3). This ratio predicts that for every percentage increase in kernel protein, the kernel should accumulate 26 mg of starch simultaneously. Therefore, with this C/N value, kernel weight and grain yield could be predicted for any hybrid at any percentage of protein produced in the kernel. N stress condition should be avoided since a higher than normal C/N ratio is induced and kernel primordia in the ear are aborted.

Most hybrids have a maximum percentage of protein attainable around 12% (Russell and Pierre 1980); although a higher value is possible, 12% represents a realistically achievable value for any given hybrid. Using the reference values obtained with B73xMo17 at 134 kg N/ha (Table 4), where the kernel weight, percent of protein and grain yield are 330mg, 9.0%, and 110q/ha, respectively, the predicted kernel weight at 12% percent protein can be calculated as follows: Since 12% protein represents an increase of 3.0% over the tested reference value of 9.0%, the predicted kernel weight is 330 mg + (26 mg x 3.0) = 408 mg. The N sink capacity can be calculated by multiplying kernel weight with percent protein. The N sink capacity at 9% and 12% protein are is 29.7 mg/kernel (330 mg x 9.0%) and 49.0 mg/kernel (408 mg x 12%), respectively. The conversion factor is determined by dividing the N sink capacity at 12% by that at 9.0% i.e. 49.0/29.7 which gives a factor of 1.65. The predicted maximum yield (at 12% protein) of this hybrid is, therefore, equivalent to 182 q/ha or 110 q/ha x 1.65. Grain yield predicted for any level of protein from the reference values obtained with various levels of N fertilizer, except zero N, agree very well with yields actually observed for B73xMo17 (Table 4). Evaluation of all 16 hybrids under various levels of N fertilizer further support this conclusion. The equation for predicting grain yield at any percentage of protein for a given hybrid is:

$$Y_2 = Y_1 [(RP_2 - RP_1 + W_1) P_2/W_1P_1].$$

Where Y_2: Predicted yield at a given percent protein.

Y_1: Reference yield value observed at a given N level

W_1: Reference kernel weight observed at a given N level

P_1: Reference protein percent observed at a given N level

P_2: Expected protein percent

R: C/N ratio of the given hybrid

The C/N ratio is not only important for predicting kernel weight and grain yield, but may also be used as a selection index for high yielding varieties. Because the C/N ratio predicts the amount of starch that can be produced with each percentage protein increased, the selection of hybrids with higher C/N ratio should yield better than hybrids with lower C/N ratios. High-yielding hybrids have a high C/N ratio and a small amount of zein (or low percentage of total

protein); therefore, the N sink (zein protein) will continue to increase in response to N fertilization and result in a greater kernel weight until N sink saturation is achieved (or when total protein in the kernel approaches 12%). Low-yielding hybrids, on the other hand, have a low C/N ratio and large quantities of zein (or a high percentage of total protein). They not only have less C movement into the kernel for every unit of N but also reach N sink saturation rapidly with only a small amount of N fertilizer. This explains why, in general, grain yields in maize are usually negatively correlated with protein content (Dudley et al., 1977), although for a given hybrid, the grain yield induced by N fertility is highly correlated with its zein or protein content (Tsai et al., 1983; 1984). A simple extrapolation of this information indicates that the percent grain protein may serve as a parameter for estimating amounts of N fertilizer required for maximum yield of a given hybrid. Studies with these 16 hybrids support this hypothesis.

Differences among maize hybrids in nitrogen uptake and utilization

Maize hybrids not only vary in yield potential, their abilities to utilize N fertilizer are also different. We have grouped maize hybrids grown in the U.S. Corn Belt into three categories based on grain yield response to N fertilizer treatments (Tsai et al., 1984). These three categories are: 1) low N-responsive types, represented by Pioneer 3732, which perform well under N stress conditions and reach their maximum yield potential with relatively low levels of applied N; 2) Intermediate types, such as B14xOh43, which respond to moderate N rates; and 3) N-responsive types, such as B73xMo17, which respond dynamically to additional N. However, the actual level of soil N conditioning a yield response within each group may be affected by plant populations and environmental factors such as moisture and temperature.

One of the major factors that distinguishes the N-responsive type from the low N-responsive type is its ability to take up large quantities of N during the period of grain-fill (Tsai et al., 1984). When grown under 201 kg N/ha at 54,000 plants/ha, Pioneer 3732, a low N-responsive type, took up and stored about 90% of its final N content by the 10th week after planting. This hybrid tolerates low levels of N in soil during the grain-fill period which makes grain production more predictable on coarse textured soil and at high plant populations. In contrast, the higher yielding hybrid B73xMo17 at the same plant density absorbed and stored only about 65% of its final N by the 10th week after planting. Thus, B73xMo17 type hybrids benefit more than Pioneer 3732 from higher levels of N fertilizer and from inhibiting nitrification or split applications of N because of their ability to take up large quantities of N after mid-silk.

When ammonical N was applied prior to planting without inhibiting nitrification, generally results in only limited amounts of N being available for plant uptake 10 weeks after planting due to denitrification, leaching, or

volatilization losses of N (Huber et al., 1977; Nelson and Huber, 1980). When available N from soil or readily available vegetative sources are not sufficient to meet the requirements of developing kernels, the plant may cannibalize enzymatic and structural proteins as sources of N. Thus, to compensate for shortage of N during grain-fill, N-responsive hybrids could conceivably cannibalize more N components from leaf and stalk tissues than low N-responsive types with the ultimate result being less than maximal yield performance and the predisposition of weakened stalks to stalk rot.

To test the hypothesis that the kernel-sink induced nutrient deficiency of N-responsive hybrids may result in cannibalization of N components, maize hybrids Pioneer 3732, B37xW64A, and B73xMo17 (representing different maturation dates and a range in yield potential) were grown at the Purdue University Agronomy Farm in 1984 on field plots which had been spring treated prior to planting with either 134 kg N/ha or 402 kg N/ha plus 0.5 kg nitrapyrin. These two N treatments were chosen for the studies to accentuate the N effect. The experimental design was a randomized complete block with three replicates. Ear-leaf and kernel samples were collected at mid-silk, 15, 30, 45, 50, 55 and 60 days post-pollination. Collections at 5-day intervals for stages beyond 45 days post-pollination were necessary to determine the time of RuBP carboxylase cannibalization and its effect on the kinetics of kernel dry matter accumulation. Whole plant tissue was collected at the mid-silk stage and at maturity for N analysis. At each sampling, ear leaves were frozen in liquid N immediately after collection, stored at -80°C before measurement of RuBP carboxylase activity (Evans and Seemann, 1984), and analyzed for cannibalization of proteins on slab gels by an SDS-polyacrylamide gel electrophoresis system similar to that of Laemmli (1970). Whole plant tissues, including leaves and stalk, were air-dried at 40°C, ground to a powder and analyzed for total N. N content was multiplied by a factor of 6.25 to convert it to percent protein. Kernels were oven-dried at 103°C prior to determining dry weight.

The literature is replete with evidence for the redistribution of N from vegetative tissues to the kernel sink during the period of grain-fill. Variation in N translocation from vegetative tissues to grain are genetically controlled (Below et al., 1981; Swank et al., 1982; Anderson et al., 1985). Protein concentrations in leaf tissues of the three hybrids tested, Pioneer 3732, B37xW64A, and B73xMo17 decreased from the mid-silk stage to maturity (Table 5). B73xMo17, a N-responsive or high-yielding hybrid, had the least amount of protein remaining in the leaf tissue at maturity since vegetative proteins were extensively cannibalized. Pioneer 3732 and B37xW64A are low N-responsive and intermediate types, respectively, and had more proteins remaining in the tissues than B73xMo17. Because nitrapyrin is an effective nitrification inhibitor in the Corn Bely (Huber et al., 1977; Nelson and Huber, 1980), application of 402 kg N/ha with nitrapyrin should provide available N for uptake during the grain-fill period and minimize cannibalization of enzymatic and structural N components in the tissues. The greater amount of protein retained in tissues at maturity with the treatment confirms this hypothesis.

RuBP carboxylase, a key enzyme responsible for CO_2 fixation, is the most abundant protein in leaves and a potential source of N for kernel development. The degree of RuBP carboxylase cannibalization generally varies inversely to the availability of N. The RuBP carboxylase activity of B73xMo17 decreased rapidly (Table 6) as the enzyme was hydrolyzed about 45 days post-pollination in insufficiently N fertilized plants (Tsai et al., 1991). This destruction of RuBP carboxylase from leaf tissues of N-responsive hybrids due to N stress during grain-fill resulted in early termination of dry matter accumulation and prevented a further increase in kernel weight and grain yield (Table 7). The highest level of available N (402 kg N/ha) stabilized with nitrapyrin evaluated in this study delayed the hydrolysis of RuBP carboxylase and other proteins, and enzyme activity was maintained up to 55 days post-pollination (Tsai et al., 1991). Accordingly, the duration of kernel dry matter accumulation was prolonged beyond that observed with the lower N treatment. These results are consistent with an observation that continuous N input into the plant and maintenance of N in the leaf tissue are important for continuing photosynthetic activity (Christensen et al., 1981; Swank et al., 1982).

The low N-responsive hybrid, on the other hand, maintained RuBP carboxylase activity throughout the 45 days post-pollination period (Table 6) at which time dry matter accumulation in the kernel was complete (Table 7). Although this enzyme can be preserved in low N-responsive hybrids with higher levels of N, no beneficial result is obtained because the requirement of N in the kernel for dry matter accumulation terminates at 45 days post-pollination. These studies clearly demonstrate that there are wide variations in hybrid response to N fertilization; and maintenance of N sufficiency throughout the crop season is critical to preserve RuBP carboxylase activity, strengthen stalk quality, and maximize grain production for N-responsive type hybrids such as B73xMo17.

Genetic variations in ability of maize inbreds to utilize ammonium nutrition

The fact that certain hybrids require N uptake throughout the growing season clearly suggests an important role of NH_4^+ nutrition for maize grain production. Although NH_4^+-N is the most prevalent form of N fertilizer applied for maize, it may be rapidly converted to NO_3^- in the soil because of nitrification. NO_3^- is susceptible to leaching and denitrification losses; therefore, the pre-plant applied N may not be available for plant utilization during the period of grain-fill. N deficiency during grain-fill may result in the early termination of dry matter accumulation due to an extensive proteolysis of leaf proteins including RuBP carboxylase (Tsai et al., 1991). Stephenson et al. (1969) reported that 50% of the added N was evolved. Thus, if NO_3^--N is not assimilated by plants or immobilized by microorganisms, it may be lost through leaching and denitrification (Prasad et al., 1971). In contrast to NO_3^-, NH_4^+ is held in soil by the cation exchange complex from which it is released slowly

into the soil solution for uptake by plants. The availability of nitrification inhibitors to reduce these losses provides an additional approach to increase the efficient utilization of nitrogen in crop production. As the concern over $N0_3^-$ pollution increases, NH_4^+ nutrition will undoubtedly become more important for crop production especially when high efficient nitrification inhibitors are being developed as N management tools.

In spite of the high efficiency of NH_4^+ nutrition for crop production, maize hybrids may not perform well under NH_4^+-N exclusively. This is because breeding programs and selection have been conducted under predominantly $N0_3^-$-N environments that could result in eliminating the trait of high efficiency in NH_4^+ assimilation. Therefore, as a first step towards developing maize hybrids capable of performing under NH_4^+-N, germplasms were evaluated for the efficiency of NH_4^+ assimilation which includes activities of NH_4^+ assimilating enzymes, photosynthetic enzymes such as PEP carboxylase, and the ability of NH_4^+ uptake.

The efficiency of NH_4^+ assimilation via the glutamine synthetase (GS)/glutamate synthase (GOGAT) and glutamate dehydrogenase (GDH) systems may be key regulatory steps enhancing N utilization and CO_2 assimilation in maize. When maize plants are grown in the presence of a nitrification inhibitor, the level of NH_4^+ may increase substantially - especially during early stages of growth. Unlike $N0_3^-$, NH_4^+ is relatively toxic at low concentrations *in vivo* (Anderson and Done, 1977) and must be assimilated immediately in the root by the reductive amination of an available carboxylic acid to form a non-toxic amino acid prior to translocation (Ivanko and Ingversen, 1971). The NH_4^+ directly absorbed or available through reduction of $N0_3^-$ is assimilated into organic N either by 1) the reductive amination of α-ketoglutaric acid by GDH to form glutamic acid or 2) by conjugation with glutamic acid to form glutamine by GS. The amide amino group of glutamine is then transferred to α-ketoglutaric acid to form glutamic acid by GOGAT (Miflin and Lea, 1977). The GS/GOGAT system is considered as the major pathway for NH_4^+ assimilation. However, based on a determination of NH_4^+ assimilation in *in vitro* and the calculation of organic N accumulated in the shoot after seedling growth on NH_4^+, our studies indicate that GS/GOGAT system alone was not sufficient to account for all of the organic N transported to the shoot (Handa et al., 1985). Thus, one cannot rule out the possibility that GDH may play an important role in NH_4^+ assimilation in the root, especially when plants are grown under high concentrations of NH_4^+.

A previous study has shown that 10 mM NH_4^+ provides a near optimum N condition for seedling development (Xu et al., 1992). Therefore, maize inbreds were grown at 10 mM and a high concentration of 50 mM NH_4^+-N to evaluate for their sensitivities of seedling growth to NH_4^+ nutrition. Of the 41 inbred lines screened, only two inbreds grew vigorously with 50 mM NH_4^+-N (more than 70% dry weight of the 10 mM control). Thirty-two inbreds grew very poorly with 50 mM NH_4^+ as the only source of N (less than 33% of the 10 mM control)

(Wu et al., 1998).

Va26, A661, H95, Hi31, Pi6875-370, ICAL210, PH9, CI66, A632, TA85-17, and Pi 77-4239, which represent different degrees of sensitivity to NH_4^+-N, were selected for further growth analysis under mixtures of NH_4^+-N and $N0_3^-$-N. With the exception of A632, all other 10 inbreds studied preferred a high proportion of N in the $N0_3^-$ form (66% or 100% $N0_3^-$-N)(Table 8). These observations are expected because of breeding programs and selection under predominantly $N0_3^-$ environments which might have inadvertently eliminated the trait for efficient NH_4^+ utilization. The finding that certain inbreds such as A632 could tolerate high proportion of N in NH_4^+ form for growth is important for developing maize hybrids which can capitalize on the efficiency of NH_4^+-N for high yield production and thereby permit the greater use of management practices, such as inhibiting nitrification, to minimize potential $N0_3^-$ pollution.

Because NH_4^+ assimilation involved three components: organic carbon skeletons (derived from PEP and RuBP carboxylase activities), assimilating enzymes (GDH, GS, and GOGAT), and NH_4^+ uptkake, efforts were made to determine which components are responsible for the ability or inability of maize inbreds to utilize NH_4^+ nutrition. As shown in Table 9, the correlation coefficient between dry weight accumulation and the activity of GS (r^2= 0.09), GOGAT (r^2= 0.004), GDH (r^2=0.016), or PEP carboxylase (r^2= 0.24) among the 11 inbreds studied was very low. Likewise, there was no correlation between dry weight and NH_4^+ uptake (r^2= 0.05, Table 10). From these data, it is clear that no one component is entirely responsible for enhanced seedling growth with high NH_4^+ levels and that a more complex interaction between enzymes exists. In other words, certain inbred lines retain the ability to effectively utilize ammonium, but adapt various combinations of mechanisms to cope with NH_4^+ pressure and thereby allow seedling growth and development in the presence of NH_4^+ in high concentrations. Therefore, plant breeders should be able to exploit this variability and create hybrids with enhanced performance under predominantly NH_4^+-N conditions. A preliminary study with maize hybrid Tainan 17 and its inbred parents supports this hypothesis. In addition, a field experiment indicated that crosses that involved the Mo17 family responded positively to inhibiting nitrification (Tsai and Huber, 1996) which provided a larger portion of the N in the ammonium form. These studies support the notion that grain yield of maize could be greatly increased through N management and selection for hybrids that are efficient for NH_4^+-N utilization.

Literature Cited

Anderson, E. L., E. J. Kamprath, and R. H. Moll. 1985. Prolificacy and N fertilizer effects on yield and N utilization in maize. Crop Sci. 25:598-602.

Anderson, J. and J. Done. 1977. A polarographic study of ammonia assimilation by isolated chloroplasts. Plant Physiol. 60:504-508.

Below, F. E., L. E. Christensen, A. J. Reed, and R. H. Hageman. 1981. Availability of reduced N and carbohydrates for ear development of maize. Plant Physiol. 68:1186-1190.

Christensen, L. E., F. E. Below, and R. H. Hageman. 1981. The effects of ear removal on senescence and metabolism of maize. Plant Physiol. 68:1180-1185.

Dudley, J. W., R. J. Lambert, and I. A. de la Roche. 1977. Genetic analysis of crosses among corn strains divergently selected for percent oil and protein. Crop Sci. 17:111-117.

Evans, J. R. and J. R. Seemann. 1984. Differences between wheat genotypes in specific activity of ribulose-1,5-bisphosphate carboxylase and the relationship of photosynthesis. Plant Physiol. 74:759-765.

Handa, S., D. M. Huber, H. L. Warren, and C. Y. Tsai. 1985. Nitrogen nutrition and N-assimilation in maize seedlings. Can. J. Plant Sci. 65:87-93.

Huber, D. M., H. L. Warren, D. W. Nelson, and C. Y. Tsai. 1977. Nitrification inhibitors - new tools for food production. BioSci. 27:523-529.

Ivanko, S. and J. Ingversen. 1971. Investigation on the assimilation of nitrogen by maize roots and the transport of some major nitrogen compounds by xylem. Physiol. Plant. 24:355-362.

Laemmli, U. K. 1970. Cleavage of structural protein during the assembly of the head of bacteriophage T4. Nature 227:680-684.

Lambert, R.J., D.E. Alexander, and J.W. Dudley. 1969. Relative performance of normal and modified protein (opaque-2) maize hybrids. Crop Sci. 9:242-243.

Lee, L. and C. Y. Tsai. 1985. Effects of sucrose accumulation on zein synthesis in maize starch-deficient mutants. Phytochemistry 24: 225-229.

Lyznik, L. A., J. Kanabus, and C. Y. Tsai. 1989. L-Glutamine and D-glucose uptake by developing endosperms of maize. Phytochemistry 28: 3271-3275

Miflin, B. J. and P. J. Lea. 1977. Amino acid metabolism. Ann. Rev. Plant Physiol. 28:299-329.

Nelson, D. W. and D. M. Huber. 1980. Performance of nitrification inhibitors in the Midwest (East). ASA Special Pub. 38:75-88.

Prasad, R., G. B. Rajale, and B. Lakhdive. 1971. Nitrification retarders and slow release nitrogen fertilizers. Advan. Agron. 23: 337-383.

Russell, W. A. and W. H. Pierre. 1980. Relationship between maize single crosses and their parent inbred lines for N content in the grain. Agron. J. 72:363-369.

Stephenson, R. C., J. R. Burford, and D. J. Greenland. 1969. Plant nutrients in soils: gaseous losses of nitrogen from soils. Biennial Report of Waite Agricultural Research Institute, South Australia (1968-69).

Swank, J. C., F. E. Below, R. J. Lambert, and R. H. Hageman. 1982. Interaction of carbon and nitrogen metabolism in the productivity of maize. Plant

Physiol. 70:1185-1190.

Tsai, C. Y., D. M. Huber, and H. L. Warren. 1978. Relationship of the kernel sink for N to maize productivity. Crop Sci. 18:399-404.

Tsai, C. Y., D. M. Huber, and H. L. Warren. 1980. A proposed role of zein and glutelin as N sink in maize. Plant Physiol. 66:330-333.

Tsai, C. Y., H. L. Warren, D. M. Huber, and R. A. Bressan. 1983. Interaction between the kernel N sink, grain yield and protein nutritional quality of maize. J. Sci. Food Agric. 34:255-263.

Tsai, C. Y. 1983. Genetics of storage protein in maize. Plant Breeding Rev. 1:103-138.

Tsai, C. Y., D. M. Huber, D. V. Glover, and H. L. Warren. 1984. Relationship of N deposition to grain yield and N response of three maize hybrids. Crop Sci. 24:277-281.

Tsai, C. L. and C. Y. Tsai. 1990. Endosperm modified by cross-pollinating maize to induce changes in dry matter and nitrogen accumulation. Crop Sci. 30: 804-808.

Tsai, C. L., I. Dweikat, and C. Y. Tsai. 1990. Effects of source supply and ink demand on the carbon and nitrogen ratio in maize kernels. Maydica 35: 391-397.

Tsai, C. Y., D. M. Huber, H. L. Warren, and A. Lyznik. 1991. Nitrogen uptake and redistribution during maturation of maize hybrids. J. Sci. Food Agric. 57: 175-187.

Tsai, C. Y., I. Dweikat, D. M. Huber, and H. L. Warren. 1992. Interrelationship of N nutrition with maize grain yield, N use efficiency, and grain quality. J. Sci. Food Agric. 58: 1-8.

Tsai, C. Y. and D. M. Huber. 1996. Genetic Variations of Maize Hybrids in grain yield Response to Potassium and Inhibiting Nitrification. J. Sci. Food Agric. 70: 263-270

Wu, H. R., G. J. Shieh, H. S. Lu, and C. Y. Tsai. 1998. Genotypic variations of maize seedlings in utilizing ammonium nutrition. Taiwania 43: 261-271.

Xu, Q. F., C. L. Tsai, and C. Y. Tsai. 1992. Interaction of potassium with the form and amount of nitrogen nutrition on growth and nitrogen uptake of maize. J. Plant Nutrition 15: 23-33.

Zink, F. 1980. Preliminary results concerning the N-assimilation in the maturing kernel of maize hybrids with different protein content. In Improvement of Quality Traits of Maize for Grain and Silage Use. W. G. Pollmer and R. H. Phipps, eds. Martinus Nijhoff Publishers.

Table 1. Osmotic potential and movement of radioactive sugars into 39-day-old endosperms of normal and *o2* kernels obtained from B14*o2*xB37 plant grown with 201 kgN/ha.

Genotype	Osmotic potential	Sugars incorporated
	x 10^5 Pa	cpm
Normal	- 4.4[a]	36,450
o2	- 8.2[b]	31,340

Data adapted from Tsai et al. (1980; 1983).
Means followed by the same letter in each column are not significantly different at 5% level of probability.

Table 2. Kernel dry weight, percent protein and grain yield of maize hybrids Pioneer 3732 and B73xMo17 supplied with different levels of N fertilizer[1].

	Pioneer 3732			B73xMo17		
N level	Dry weight	Protein	Yield	Dry weight	Protein	Yield
KgN/ha	mg/kernel	%	q/ha	mg/kernel	%	q/ha
0	302[a]	8.8[a]	43.4[a]	293[a]	7.3[a]	27.3[a]
67	370[b]	11.1[b]	83.7[b]	312[a]	8.2[b]	94.2[b]
134	368[b]	10.9[b]	88.0[b]	330[b]	9.0[c]	109.7[c]
201	370[b]	11.5[b]	92.4[b]	347[bc]	9.5[cd]	123.4[d]
268	369[b]	11.5[b]	90.5[b]	349[bc]	9.4[cd]	129.0[d]
447	372[b]	11.4[b]	93.6[b]	351[c]	9.8[d]	130.8[d]

Data from 1980.
Means followed by the same letter in each column are not significantly different at 5% level of probability.

Table 3. Grain yields and C/N ratios of various maize hybrids evaluated at 67, 134, 201, and 447 kgN/ha.

Hybrid	Grain yield 67	134	201	447	C/N
	----------	q/ha	----------		Ratio
B73xMo17	102	115	123	127	26
Mo17xB73	116	120	123	127	26
H99xB14	96	113	117	119	23
B14xH99	89	117	114	123	23
Pioneer 3732	82	84	89	87	23
Va26xB14	77	95	106	110	22
B14xVa26	87	96	107	107	22
B14xB37	69	80	97	106	22
Pioneer 3780	92	97	96	99	21
B14xOh43	86	92	90	102	21
Oh43xB14	78	95	96	102	21
A545xB14	62	95	102	103	19
B14xA545	78	96	96	99	19
W64AxB37	66	81	86	85	19
B37xW64A	66	76	80	85	19

Data from 1979 and 1980. BLSD = 11 q/ha.

Table 4a. Observed values for kernel weight, % protein and grain yield of B73xMo17.

N level	Kernel wt.	Protein		Starch	C/N	Grain yield
Kg/ha	mg/kernel	%	mg/kernel	mg/kernel	ratio	q/ha
0	293[a]	7.3[a]	21.4	205	28	27[a]
67	312[a]	8.2[b]	25.6	218	27	94[b]
134	330[b]	9.0[c]	29.7	231	26	110[c]
201	347[bc]	9.5[cd]	33.0	243	26	123[d]
268	349[bc]	9.4[cd]	32.8	244	26	129[d]
447	351[c]	9.8[d]	34.4	246	25	131[d]

Data from 1980.
Means followed by the same letter in each column are not significantly different at 5% level of probability.

Table 4b. Predicted and observed grain yields of B73xMo17 at various percentage of protein obtained under different levels of N fertilizer.

	Predicted yield using following % of protein						Observed
N level	7.3	8.2	9.0	9.5	9.4	9.8	Yield
Kg/ha	----- predicted grain yield (q/ha) ----						q/ha
67	33	---	94	96	103	97	94
134	38	110	---	112	120	113	110
201	42	121	121	---	132	124	123
268	41	118	118	121	---	122	129
447	44	127	127	130	138	---	131
Maximum	63	181	182	184	197	186	

Table 5. Protein concentration in the leaf tissue of maize hybrids fertilized with two levels of N fetilizer.

Hybrid	Stage	134 kgN/ha	402 kgN/ha*
		--------- % protein ---------	
Pioneer 3732	Mid-silk	15.8 ± 1.7	19.3 ± 3.4
	Maturity	6.4 ± 0.9	8.8 ± 0.7
B37xW64A	Mid-silk	16.0 ± 0.4	19.4 ± 2.0
	Maturity	6.6 ± 0.9	9.5 ± 0.5
B73xMo17	Mid-silk	13.8 ± 1.2	17.0 ± 1.8
	Maturity	5.9 ± 0.9	7.1 ± 0.6

*Stabilized with 0.55 kg nitrapyrin/ha
Data adapted from Tsai et al. (1991).

Table 6. RuBP carboxylase activity in maturing ear leaves of Pioneer 3732 and B73xMo17 fertilized with two levels of N fertilizer.

			Days after pollination					
Hybrid	N level	Midsilk	15	30	45	50	55	60
	Kg/ha	---------- nCi incorporated/g. fresh weight ----------						
Pioneer 3732	134	256	256	312	304	60	0	0
	402*	595	812	926	450	472	438	0
B73xMo17	134	498	394	600	212	24	18	0
	402*	376	550	513	335	326	286	0

*Stabilized with 0.55 kg nitrapyrin/ha
Data adapted from Tsai et al. (1991).

Table 7. Kernel dry weight during development and grain yield of Pioneer 3732 and B73xMo17 fertilized with two levels of N fertilizer.

Hybrid	N level	Days after pollination						Grain yield
		15	30	45	50	55	60	
	Kg/ha	----------		mg/kernel		----------		q/ha
Pioneer 3732	134	30	174	297	276	280	304	99
	402*	31	167	290	298	305	306	101
B73xMo17	134	35	172	289	303	315	321	105
	402*	40	186	304	331	369	370	124

*Stabilized with 0.55 kg nitrapyrin/ha
Data adapted from Tsai et al. (1991).

Table 8. The effect of N forms (30 mM) on total seedling dry matter accumulation in 11 maize inbreds.

Inbreds	NH_4^+ (mM)/NO_3^- (mM)			
	30/0	20/10	10/20	0/30
	----------	Dry weight/seedling		----------
Va26	0.76	1.07	1.23	1.21
Pi6875-370	1.46	1.75	2.15	1.70
C166	0.99	1.42	1.79	1.75
H95	1.39	1.66	2.22	1.94
A661	1.28	2.23	2.06	1.94
PH9	0.73	0.86	1.06	1.09
A632	0.92	0.98	1.29	1.20
Hi31	0.62	1.17	1.14	1.49
Pi77-4239	0.60	1.07	1.15	1.63
TA85-17	0.39	0.65	0.83	0.81
ICAL210	0.63	0.95	1.20	1.21

Data adapted from Wu et al. (1998).

Table 9. Relationship between seedling dry weight and enzyme activity of NH_4^+ assimilation and PEP carboxylase in 11 maize inbreds.

Genotype	Dry weight	GS	GOGAT	GDH	PEPcase
	g/seedling	---------- unit/mg protein/min ----------			
Va26	0.84	0.67	0.018	0.015	0.29
Pi6875-370	0.66	0.93	0.038	0.021	0. 19
C166	0.64	0.83	0.073	0.032	0.55
H95	0.52	1.30	0.071	0.021	0.39
A661	0.51	0.83	0.018	0.036	0.35
PH9	0.51	0.52	0.021	0.019	0.65
A632	0.43	0.97	0.049	0.034	0.22
Hi31	0.37	0.33	0.051	0.026	0.64
Pi77-4239	0.34	0.24	0.040	0.023	---
TA85-17	0.30	0.67	0.009	0.028	0.51
ICAL210	0.30	0.44	0.050	0.031	0.49

Data adapted from Wu et al. (1998).

Table 10. Relationship between NH uptake activity and seedling dry weight of 11 maize inbreds.

Genotype	Dry weight	Uptake
	g/seedling	u mol/g FW/h
Va26	0.84	2.25
Pi6875-370	0.66	2.30
C166	0.64	1.43
H95	0.52	1.45
A661	0.51	4.25
PH9	0.51	2.67
A632	0.43	1.26
Hi31	0.37	2.90
Pi77-4239	0.34	1.64
TA85-17	0.30	1.56
ICAL210	0.30	0.31

Data adapted from Wu et al. (1998).